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Chemoreception, symmetry and mate choice in lizards

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Research on fluctuating asymmetry (FA)-mediated sexual selection has focused almost exclusively on visual signals and ignored chemical communication despite the fact that many species rely on chemical signals for attracting mates. Female mate choice based on visual traits appears to be rare in lizards. However, the femoral glands of male lizards produce pheromones which might transmit chemical information about an individual's developmental stability. Therefore, we hypothesized that mate choice may be based on chemical cues. We analysed the effect of the developmental stability levels of males on the attractiveness of males' scents to females in a laboratory experiment with the lizard *Lacerta monticola*. When we offered two males of similar body size, females preferentially associated with the scents of males with low FA in their femoral pores and also with the scents of males with a higher number of femoral pores. This suggested that the females were able to discriminate the FA of the males by chemical signals alone and that the females preferred to be in areas marked by males of high quality, thus increasing their opportunities of mating with males of high quality. We suggest that the quality and/or amount of male pheromones could communicate the heritable genetic quality of a male to the female and thereby serve as the basis for adaptive female choice in lizards.

Keywords: chemoreception; fluctuating asymmetry; femoral glands; mate choice; lizards

1. INTRODUCTION

Fluctuating asymmetry (FA) is random deviations from perfect symmetry which arise because of the inability of individuals to undergo identical development of bilaterally symmetrical traits on both sides of the body (Van Valen 1962). Low levels of FA are preferred in a potential mate in many species (Møller & Thornhill 1998) because they presumably indicate the developmental stability of an individual and, thus, its ability to cope with genetic and environmental perturbations during development (Møller & Swaddle 1997). Although communication by chemical signals is widespread (Stoddart 1980), research on FA-mediated sexual selection has focused almost exclusively on visual signals and ignored chemical communication (Møller & Thornhill 1998; but see Thornhill 1992; Gangestad & Thornhill 1998; Rikowski & Grammer 1999). However, many species rely on chemical signals for attracting mates and male odours convey a great deal of information, such as a male's dominance status, health, parasite load or genetic quality (e.g. Moore *et al.* 1997; Penn & Potts 1998).

Chemical cues also play an important role in the intraspecific communication of lizards (Halpern 1992; Mason 1992; Cooper 1994). Several studies have shown pheromonal detection in different species, which is often based on pre-cloacal and femoral gland secretions (e.g. Cooper & Vitt 1984; Alberts 1989; López *et al.* 1998). The femoral pores are epidermal structures on the ventral surface of the thigh of many squamates which have been extensively used in taxonomy, but little is known about their functional significance (Alberts 1993). They are connected to glands that are formed by an invagination of the stratum germinativum, which forms a follicular unit, and produce copious amounts of holocrine secretion (Mason 1992). The secretory activity of the femoral glands is greatest in the breeding season; males produce more secretions than females and androgens can influence their development and maintain their activity (Cole 1966; Van Wyk 1990; Alberts 1993). Moreover, the presence and relative concentrations of the pheromone components vary not only between sexes but also consistently between individuals, which may convey information about individual identity and serve a variety of functions (Alberts 1990, 1992, 1993). Female choice based on visual quantitative traits has rarely been demonstrated in reptiles (Olsson & Madsen 1995; Tokarz 1995). However, we hypothesized that femoral pore secretions might transmit chemical information about an individual's developmental stability and that this information may be used by female lizards in their mate choice process.

The Iberian rock lizard (*Lacerta monticola*) is a small diurnal lacertid lizard found mainly in the rocky habitats of some high mountains of the Iberian Peninsula (Martín & Salvador 1992). Male *L. monticola* deposit faecal pellets and femoral secretions on specific sites and are able to detect and discriminate individuals based on chemical cues alone (López *et al.* 1998; P. López, P. Aragón and J. Martín, unpublished data). Substrates marked with faeces and femoral gland secretions may serve as scent marks in the field and probably also function in home range advertisement (Alberts 1992; López *et al.* 1998). Males are aggressive, many are territorial, although overlap between home ranges is extensive and male territories can contain several female home ranges (Martín & Salvador 1993, 1997). One advantage of chemical signals is that they can be used to obtain information about an individual even when other sensory cues are absent. Thus, females might choose where to establish their home ranges by relying on information coming from the chemical signals left from territorial males.

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We designed a laboratory experiment in order to analyse the effect of the developmental stability levels of *L. monticola* males on the attractiveness of their scents to females. We show that, when we offered the scents of two males of similar body size, females preferentially associated with the scents of males with low FA in their femoral pores (the structure which produce pheromones), which suggests that females are able to discriminate the FA of males by chemical signals alone and that females prefer to be in areas marked by males of high quality.

2. METHODS

(a) *Study animals*

We captured 20 adult male and 18 adult female *L. monticola* by noosing in different locations over a large area during May 1999 (Puerto de Navacerrada, Guadarrama Mountains, Central Spain) in order to ensure that individuals had not been in previous contact, which may have affected the outcome of the experiment. The lizards were weighed and their snout–vent length (SVL) was measured (means ± s.e.s) (males, SVL = 73.7 ± 0.4 mm, range = 71–77 mm, and body mass = 7.8 ± 0.2 g, range = 6.5–9.5 g; females, SVL = 74.7 ± 1.2 mm, range = 68–86 mm, and body mass = 6.3 ± 0.3 g, range = 4.5–9 g). They were housed individually at El Ventorrillo field station (Navacerrada, Madrid Province) 5 km from the capture site in outdoor plastic cages (80 cm × 50 cm) containing rocks for cover. The male and female cages were in different locations to ensure that there was no previous contact between them. Food (mealworms and crickets) dusted with a multivitamin powder and water were provided *ad libitum*. The lizards were held in their home cages for at least one week in order to familiarize them with their new environment prior to testing. All the animals were healthy during the trials and at the end of the experiments were released to their initial sighting location prior to capture. The lizards were captured before the start of the mating season. We confirmed that females had not mated yet because they did not show the characteristic mating scars on their bellies. The experiments were carried out during May and June 1999, which coincided with the mating season of the lizards in their original natural populations.

(b) *Measurement of femoral pores asymmetry*

The choice of femoral pores for measuring symmetry was not arbitrary because asymmetry might presumably be related to the quality of the pheromone output upon which females may base their choice. Thus, we considered that femoral pores may be a secondary sexual trait the asymmetry of which might affect the quality of a male for a female. We counted the number of femoral pores in the right and left hind limbs of males under a dissecting loupe (right limb = 18.2 ± 0.3 pores, range = 16–20 pores, left limb = 18.5 ± 0.3 pores, range = 16–21 pores; $n = 20$ males). The counts were repeated on three occasions and were shown to be highly repeatable. We used likelihood ratio g-tests to compare the counts in each asymmetry class versus the counts in each class of deviations between replicates in order to yield an estimate of the statistical significance of the between-sides variation relative to the counting error ($p < 0.0001$ in all cases) (see Palmer 1994). The absolute value of the asymmetry of the femoral pores was calculated as the unsigned right-minus-left number of pores and this exhibits the properties of FA, i.e. a normal distribution (Filliben correlation coefficients, i.e. correlation of the raw data with the predicted normal probability scores (Aitken *et al.* 1989), $r = 0.92$, $n = 20$ and $p < 0.0001$) around a mean of zero (one sample t-test, $t_{19} = 1.0$ and $p = 0.33$).

(c) *Choice of scent experiments*

We placed several absorbent paper strips (35 cm × 10 cm) fixed to the floor of the cage in each male's cage and left them there for ten days in order to obtain the scents and secretions from the femoral and cloacal glands of the lizards. The males were often observed depositing faeces and rubbing their cloaca and femoral pores on this paper substrate. The papers were removed for placing in the females' cages immediately before each experiment began.

The females' cages (80 cm × 50 cm) had two basking platforms (two identical flat tiles) placed symmetrically at each end of the cage and rocks for cover in the centre. At the beginning of each experiment (07.00 GMT), when the females were still inactive and hidden in the refuge, we fixed one paper strip from one male on one tile and another from a different male on the other tile. The paper strips were manipulated with fresh gloves in order to avoid contaminating them with human odours. Female choice is dependent on body size in some lizards (Cooper & Vitt 1993; Censky 1997) and, thus, to avoid the influence of this trait on the results, we classified the males into two categories of body size (SVL = 71–73 mm and $n = 12$, and SVL = 75–77 mm and $n = 8$) and used two males of similar size in each test. The pair of males tested within each category of body size and the position of the papers in the cages were determined randomly. Each female was tested on four different days with papers from eight different males (four pairs of two pairs from each category of body size). We used the instantaneous scan sampling method; females were monitored every 30 min from a hidden point recording their location in the cages. Female *L. monticola* have limited movement rates, spending long periods of time stationary and use more a sit-and-wait foraging strategy (Martín & Salvador 1997). Thus, the locations observed on each of the 17 scans were considered to be representative of females' use of space in the cages. If a female was located on either of the two tiles with the paper strip, she was designated as having chosen that particular paper, whereas if she was not located on the tiles she was designated as having made no choice. In order to ensure that females were exposed to both males' tiles and were aware of both males' stimuli, at least two recordings in each male's section were considered necessary for a trial to be valid. This presumption was fulfilled in all tests. We determined the female's choice by calculating on which paper the female spent greater than 50% of her time (excluding time spent in the no choice area). Each trial lasted 8 h (between 08.00 and 16.00 GMT, when lizards again hid in the refuge), at which time the papers were removed and the cage thoroughly rinsed with clean water.

Different paper strips from each individual male were used in between six and ten odour choice tests against the papers of others males of the same size class with different individual females. Each individual male was assigned an 'attractiveness index' score, which was calculated as the proportion of trials in which a paper impregnated with his scent was chosen by a female. There was no significant relationship between the number of times that a male was tested and his attractiveness index (Spearman's rank correlation, $r_S = -0.13$, d.f. = 19 and $p = 0.58$). We used non-parametric rank Spearman correlations and Kendall partial rank-order correlations (Siegel & Castellan 1988) for analysing the relationships between asymmetry and attractiveness because of the particular half-normal distribution

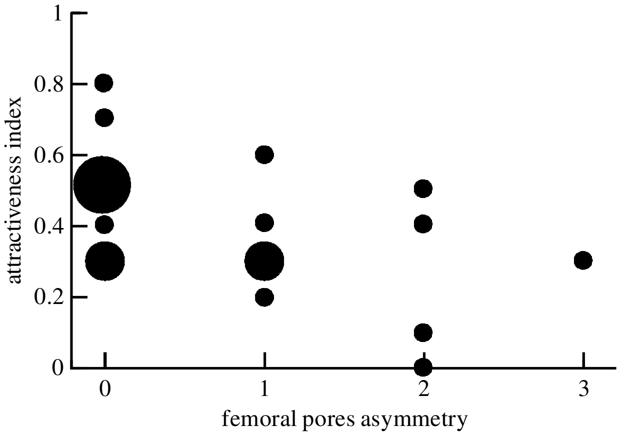

Figure 1. Relationship between the index of attractiveness of males' scent to females and the FA of femoral pores of males (the unsigned right-minus-left number of pores). Circles of increasing size represent one, two or five observations, respectively.

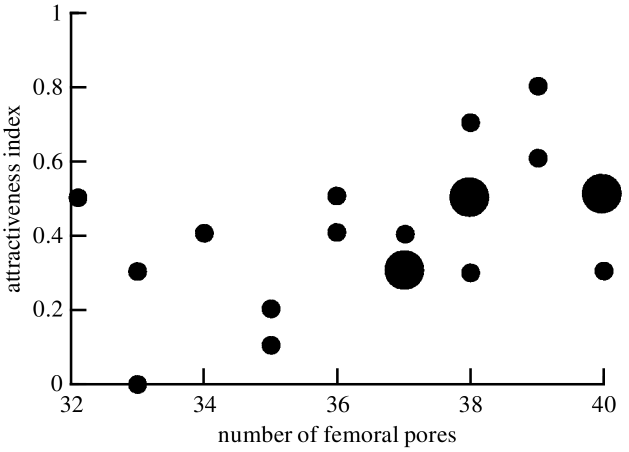

Figure 2. Relationship between the index of attractiveness of males' scent to females and the total number of femoral pores (right and left sides pooled) of males. Circles of increasing size represent one or two observations, respectively.

of the unsigned absolute asymmetry data (see Swaddle *et al.* 1994).

3. RESULTS

The attractiveness indexes were significantly and negatively correlated with the absolute value of the FA of the males' femoral pores ($r_S = -0.52$, d.f. = 19 and $p = 0.02$) (figure 1). Thus, the females preferentially associated with the scent of males with low levels of FA. In addition, the females associated more frequently with the scent of males which had a higher number of femoral pores ($r_S = 0.50$, d.f. = 19 and $p = 0.03$) (figure 2). However, we did not find a significant negative relationship between the FA of the femoral pores and the number of femoral pores ($r_S = -0.26$, d.f. = 19 and $p = 0.26$). The Kendall partial correlations showed that both FA ($\tau = -0.55$ and $p < 0.0005$) and the number of pores ($\tau = 0.47$ and $p < 0.0025$) affected the attractiveness indexes significantly and independently when the effects of the other were fixed.

As we controlled for the effects of body size in our experiment, the body size of the males did not seem to influence the choice of scent by females because the attractiveness indexes were not significantly correlated with either the SVL ($r_S = 0.04$, d.f. = 19 and $p = 0.85$) or the body mass of the donor male ($r_S = -0.15$, d.f. = 19 and $p = 0.51$). The total number of femoral pores did not change significantly with male body size when considering both the SVL ($r_S = -0.20$, d.f. = 19 and $p = 0.38$) or body mass ($r_S = -0.07$, d.f. = 19 and $p = 0.76$). Similarly, the absolute value of the asymmetry did not change significantly with body size (SVL, $r_S = -0.14$, d.f. = 19 and $p = 0.54$ and body mass, $r_S = 0.08$, d.f. = 19 and $p = 0.73$).

4. DISCUSSION

Our results show that, when we offered two males of similar body size, female *L. monticola* preferred to associate with the scents of males with low FA in their femoral pores and also with the scents of males with a higher number of femoral pores. These results suggest that female *L. monticola* selected the areas scent marked by males of high quality, as indicated by the number of pores and FA levels. Therefore, females may increase their opportunities of mating with males of high quality. Alternatively, even if females do not intend to mate, they could use males' chemical signals in selecting high-quality territories if high-quality males also maintain territories of high quality in terms of food availability and thermoregulatory opportunities.

Female mate choice appears to be rare in lizards (Olsson & Madsen 1995; Tokarz 1995), although female choice based on male body size has indeed been demonstrated in some lizard species (Cooper & Vitt 1993; Censky 1997). Moreover, bright male colours might be important in social relationships between males but they seem to be unimportant in female mate choice (Olsson 1994; Martín & Forsman 1999). Discriminations based on pheromone components might be more reliable and may also provide more detailed information about the conspecific than might be obtained from colour patterns alone (Alberts 1992). For example, testosterone stress did not increase the asymmetry of a coloured sexual ornament in the lizard *Psammodromus algirus* (Veiga *et al.* 1997), but its effect on chemical signals has not been studied. Our study suggests that mate choice in lizards may rather be based on chemical cues. However, this possibility has been largely ignored in studies of mate choice in lizards (Tokarz 1995), despite pheromonal recognition being widespread among lizards (Halpern 1992; Mason 1992).

The properties of these lizard pheromones are propitious for use in female choice. Femoral gland secretions are composed of both lipids and proteins (Alberts 1990). Lipids have a high degree of molecular diversity, which increases the potential information content of a pheromone and individual differences in protein secretion chemistry are consistent over time, which could potentially function in individual recognition (Glinsky & Krekorian 1985; Alberts 1992), but could also indicate the characteristics and quality of the male. Moreover, femoral gland secretions are principally proteinaceous and, thus, the bionergetic costs associated with their production may be high (Alberts 1993). Moreover, the production of pheromones may be costly because it depends on testosterone and an elevation of the plasma testosterone concentration affects the immune response of some lizards and renders individuals more susceptible to parasites (Salvador *et al.* 1996). Thus, the characteristics of the chemical signals arising from the femoral pores' secretion may be a reliable indicator of male quality. As it occurs in, for example, insects (Breed *et al.* 1980; Thornhill 1992), the quality or amount of male pheromone could communicate the heritable genetic quality of the male to the female and thereby serve as the basis for adaptive female choice.

Recent evidence has suggested that female lizards selecting more symmetrical males could have some advantages. First, a direct relationship between high FA and an increased susceptibility to parasitism has been convincingly demonstrated in the lizard *Sceloporus occidentalis* (Schall 1995). Thus, males with low FA may have a better immune system for defending themselves against parasite attack. Second, cold incubation temperatures induced significant developmental instability in the lizard *Sceloporus virgatus* and more asymmetrical individuals, including those showing FA in the femoral pores, also exhibited poorer quality phenotypes for other traits, such as running speed or escape performance (Qualls & Andrews 1999). Because unfavourable thermal conditions are limiting for lizards at high altitude (Carrascal *et al.* 1992), female *L. monticola* might be selecting males developed at optimal temperatures and, indirectly, the genotypes of females able to select appropriate egg-laying sites.

Our results also suggest that female *L. monticola* might be favouring an increase in the number of femoral pores in males. This structure could be considered as a secondary sexual trait which may be specially vulnerable to stress-induced developmental perturbations. An increase in the number of femoral pores and, thus, in the amount of holocrine glands which secrete pheromone components may be costly for a male. Moreover, lower developmental stability may be more difficult to obtain when the number of femoral pores increases. The regular occurrence of one or two 'supernumerary' femoral pores at the medial ends of the femoral pores series in some lizards having lengthy series which allow little space for expansion, as in *Sceloporus merriami*, may reflect selection pressures such as those inferred here. However, the pheromonal significance of femoral pores may vary considerably among species as suggested by *Sceloporus horridus*, in which only two or three pores occur on each leg, whereas all others of that genus have several times that many.

We conclude that, because most species of lizards seem to be able to detect pheromones from conspecifics (Mason 1992), female mate choice based on chemosensory cues may be more widespread than previously thought among lizards. Our findings point to the need for further studies of mate choice in lizards taking into account the different roles and the relative importance of morphological, colour and chemical cues.

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